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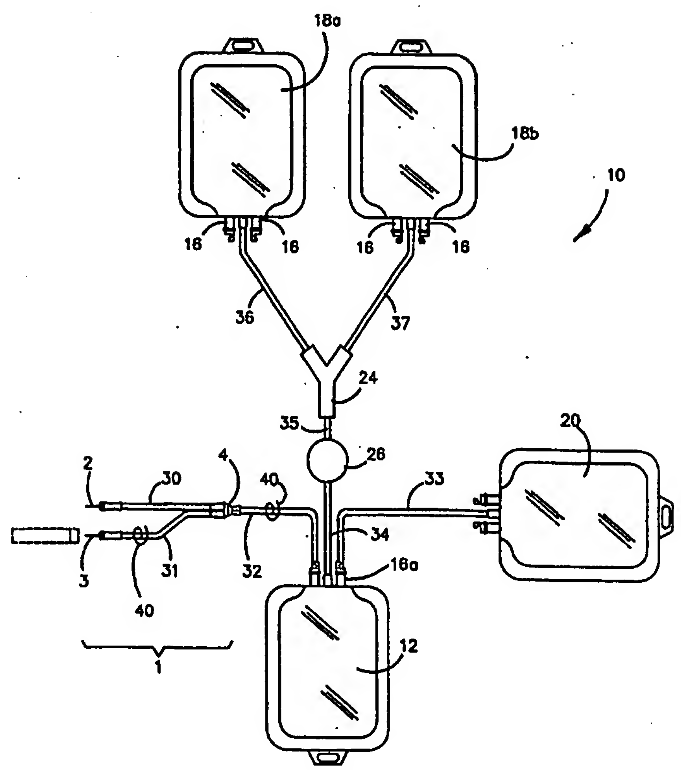
(54) Title: BIOLOGICAL FLUID PROCESSING

(57) Abstract

Methods and systems for processing biological fluid, using a phlebotomy device (1) to obtain separate portions of biological fluid, are disclosed.



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# BIOLOGICAL FLUID PROCESSING

This application claims the benefit of U.S. provisional patent application 60/034,145, filed December 24, 1996, which is incorporated by reference in its entirety.

## Technical Field

This invention relates to processing biological fluids such as blood and blood components. The invention particularly relates to removing undesirable material from the biological fluid and/or minimizing contamination of the biological fluid with the undesirable material.

## Background of the Invention

Blood consists of a number of components having different characteristics and uses. Accordingly, blood is typically processed to separate the components to yield a variety of valuable blood products. For example, a unit of donated whole blood can be processed to separate red cells, usually concentrated as packed red cells (PRC), platelets, usually concentrated as platelet concentrate (PC), and plasma. In accordance with some processing protocols, blood can be treated to form platelet-rich-plasma (PRP) or buffy coat, before forming PC and/or separating plasma.

The separated components can be stored before being used as a blood product, particularly before being used as a transfusion product. Illustratively, PC can be stored for several days or more, and PRC can be stored for several weeks or more, before transfusion into a patient. Moreover, multiple units of some components, e.g., PC, buffy coat, and/or plasma, can be pooled before producing the final blood product. Two or more units can be pooled and transfused without having been stored as individual units. Alternatively, units can be pooled and then stored before use.

However, stored and/or non-stored components typically include undesirable material such as bacteria and/or leukocytes. Bacteria can contaminate the blood or blood component during blood collection and/or storage. One source of bacterial contamination may be the blood donor's skin, which may contain one or more varieties of bacteria, e.g., gram positive bacteria such as Staphylococcus epidermidis, and S.

aureus, and/or gram negative bacteria. Since swabbing the donor's skin (e.g., with alcohol) prior to venipuncture may be inadequate to assure sterility, the bacteria may pass into the blood collection container, and the bacteria may reproduce while the blood or blood component is stored. Moreover, since some phlebotomy needles may cut a disc of skin when the phlebotomy needle is inserted into the donor, the bacteria-containing skin plug can pass with the blood into the blood collection container, and the bacteria can reproduce during storage.

Since some blood components (e.g., platelets) are typically stored at ambient temperatures, the problem of contamination may be magnified, since some bacteria reproduce more rapidly at ambient temperatures. The administration of the bacterially contaminated transfusion product, particularly when the product contains massive bacterial contamination, may have adverse affects on the recipient. As a result, the United States prohibits the transfusion of platelet products that have been stored for more than 5 days, since platelets stored for 7 days are considered more likely to have massive bacterial contamination. Japan and Europe have similar, or even stricter, prohibitions. Additionally, fear of contamination is one reason that the United States prohibits the transfusion of pooled platelet products unless the platelets are transfused within four hours of pooling.

Blood and blood components also contain other undesirable material. In particular, blood and blood components contain varying amounts of leukocytes, which may also adversely affect the recipient receiving the leukocyte containing transfusion product. For example, the administration of leukocyte contaminated transfusion products has been associated with febrile reactions, alloimmunization, and Graft Versus Host Disease. Moreover, the presence of leukocytes during blood component storage may be undesirable, as leukocytes may contain bacteria, and/or have bacteria attached thereto, and the bacteria may reproduce as noted above. Additionally, or alternatively, the leukocytes can release products that adversely affect the blood components during storage, or adversely affect the patient receiving the transfusion.

Some material present in blood is undesirable when the material is present in particular transfusion products. For example, platelet-containing transfusion products such as platelet concentrate (PC) should be substantially free of red blood cells. Since red blood cells are antigenic, the presence of a significant level of red blood cells in a

platelet transfusion product can lead to an adverse immune response by the patient. The problem is magnified when multiple units of platelets (typically 4-6 units) are pooled before transfusion, since the patient can be exposed to multiple (e.g., 4-6) sets of red blood cells, each set of cells having a different antigenicity.

Thus, there is an unaddressed need in the art to minimize the presence of undesirable material in blood or blood components, particularly in stored blood components, more particularly in stored blood products that are pooled before transfusion into a patient. Additionally, there is a need in the art to reduce the likelihood that undesirable material such as bacteria that may be present in the blood component can reproduce to a significant level during storage.

The present invention provides for ameliorating at least some of the disadvantages of the prior art. These and other advantages of the present invention will be apparent from the description as set forth below.

## Summary of the Invention

Methods and systems according to the present invention provide for minimizing the presence of undesirable material such as bacteria and leukocytes in donated biological fluid to be used for blood products, preferably by providing that a first portion of donated biological fluid (which may include bacteria passed from the donor's skin, and/or the donor's skin plug), is collected separately than a second portion of donated biological fluid (which is less likely to present a significant risk of bacterial contamination). The second portion of donated biological fluid is processed to separate one or more blood components of interest (e.g., plasma, platelets, and/or red blood cells) to produce a variety of blood products, and at least one desirable blood component is depleted of leukocytes. Preferably, the leukocyte depletion is carried in a closed system.

In some embodiments, the first and second portions of biological fluid are collected from each of a plurality of sources (e.g., blood donors), and the plurality of second portions (or blood components thereof) are subsequently pooled. The blood components can be pooled before, after, or while being depleted of leukocytes.

In some embodiments, the leukocyte depletion of the biological fluid can be carried out while minimizing contamination of the collected leukocyte depleted

biological fluid with red blood cells. Accordingly, the collected leukocyte depleted biological fluid can be substantially free of red blood cells.

Methods and systems according to the invention also provide for storing the leukocyte depleted portion of biological fluid while killing and/or preventing the reproduction of undesirable material that may be present in the biological fluid. For example, the leukocyte depleted portion of biological fluid can be stored in a container that has a bacteriocidal or bacteriostatic effect on bacteria that may be present in the biological fluid. In one illustrative embodiment according to the invention, the container comprises polyvinyl chloride (PVC) plasticized with tri (2-ethylhexyl) trimellitate (TOTM), and the leukocyte-depleted biological fluid comprises platelet-containing fluid (e.g., platelet concentrate).

Methods and systems according to the invention are particularly suitable for those protocols that include pooling of blood components, especially components such as PC or buffy coat.

Brief Description of the Drawings

Figure 1 illustrates an embodiment of a system according to the present invention, allowing the separate collection of a first portion of a biological fluid, and a second portion of a biological fluid. The illustrated system includes a leukocyte depletion filter.

Figure 2 illustrates another embodiment of a system according to the present invention, allowing the separate collection of a first portion of a biological fluid, and a second portion of a biological fluid.

Figure 3 illustrates a system for pooling biological fluid from a plurality of sources (e.g., a plurality of blood donors). The illustrated system includes a pooling assembly interposed between a plurality of biological fluid source containers and a biological fluid receiving container. Each biological fluid source container is suitable for holding a second portion of a biological fluid, or at least one blood component separated from the second portion of biological fluid.

Figure 4 illustrates embodiments of systems that are especially useful for processing blood components such as buffy coat. Figure 4A illustrates an embodiment of a system for producing buffy coat, and Figure 4B illustrates an embodiment of a

system for pooling buffy coats.

Specific Description of the Invention

In accordance with an embodiment of the invention, a method for processing biological fluid comprises obtaining a first portion of biological fluid, obtaining a second portion of biological fluid, and passing at least one component of the second portion of biological fluid through a leukocyte depletion medium.

Embodiments according to the invention comprise processing biological fluid from a plurality of sources. For example, one embodiment of a method comprises (A) obtaining a first portion of biological fluid from a first source of biological fluid, and obtaining a second portion of biological fluid from the first source of biological fluid; (B) obtaining a first portion of biological fluid from a second source of biological fluid, and obtaining a second portion of biological fluid from the second source of biological fluid; and (C) combining at least one component of the second portion of biological fluid from the first source of biological fluid with at least one component of the second portion of biological fluid from the second source of biological fluid to produce a pooled biological fluid. Embodiments of the method can also include leukocyte depleting at least one component of the second portions of biological fluid, or leukocyte depleting the pooled biological fluid.

Typically, the biological fluid is processed in a closed system. In some embodiments, the pooled or unpooled biological fluid is stored for at least two days before being used as a transfusion product. In an embodiment, the processed biological fluid is stored in a container comprising polyvinyl chloride (PVC) plasticized with tri (2-ethylhexyl) trimellitate (TOTM).

In accordance with embodiment of the invention, a system for processing biological fluid comprises a phlebotomy device including at least two needles, wherein at least one needle is suitable for penetrating the skin of a biological fluid donor, and a leukocyte depletion filter assembly in fluid communication with the phlebotomy device.

Each of the components of the invention will now be described in more detail below. Like components have like reference numbers.

Figures 1 and 2 illustrate embodiments of a biological fluid processing system 10 in accordance with the present invention. The exemplary illustrated system 10 includes

a plurality of containers 12, 18a, 18b, and 20, in fluid communication with a plurality of conduits 32-37, a plurality of connectors 4 and 24, a filter assembly 26 (Figure 1), and a phlebotomy device 1. The system 10 also includes at least one, and typically two or more flow control devices 40. In some embodiments (not shown), the system includes at least one additional filter assembly, e.g., interposed between containers 12 and 20. Typically, as noted in more detail below, container 12 is suitable for receiving a unit of biological fluid, which can be processed to form a supernatant layer including platelet-rich-plasma, and a sediment layer including red blood cells; or processed to form a supernatant layer including platelet-poor-plasma, an intermediate layer including the buffy coat, and a sediment layer including red blood cells.

The phlebotomy device 1 illustrated in Figures 1, 2 and 4A comprises a connector 4, a plurality of conduits 30 and 31, and a plurality of needles 2 and 3. At least one of the needles is a phlebotomy needle. In an embodiment, needles 2 and 3 are both phlebotomy needles. The illustrated device 1 can be pre-assembled before connection to the other components of the system.

Of course, the phlebotomist device can have other configurations, e.g., additional conduits, connectors, and/or needles.

The phlebotomy device 1 includes at least one needle suitable for penetrating the skin of a blood donor, and the device 1 is suitable for obtaining a plurality of portions of biological fluid so that:

(1) at least the first portion is not passed into biological fluid receiving container 12, and

(2) a subsequent portion is passed into the receiving container 12.

Since it is believed that the first portion of biological fluid presents an increased potential for bacterial contamination, preventing the passage of the first portion into receiving container 12 as described below allows a subsequent portion to be collected in container 12 that is less likely to present the risk of significant bacterial contamination.

Using the embodiment illustrated in Figure 1 for reference, needle 2 is suitable for penetrating the skin of a blood donor, and needle 3 (which can be identical to needle 2) is suitable for penetrating the cap of a blood sampling device such as a vacutainer (shown in dotted lines). With flow control device 40 associated with conduit 31 open, and flow control device 40 associated with conduit 32 closed (to prevent fluid

communication with the biological fluid receiving container 12), such an arrangement can allow a first portion of a biological fluid such as blood to pass from the donor, through needle 2, conduit 30, conduit 31, and needle 3 into the blood sampling device. In some embodiments, this first portion of a biological fluid is collected essentially free of anticoagulant in the sampling device.

Subsequently, i.e., after closing the flow control device 40 associated with conduit 31 and opening flow control device 40 associated with conduit 32, a second portion of biological fluid (which is less likely to present the potential for significant bacterial contamination) can be passed into container 12. The second portion can be further processed, e.g., to separate blood components and to leukocyte deplete at least one separated component, as described below.

In some embodiments, blood components are separated from each of a plurality of second portions (e.g., from different donors), and the blood components are pooled. For example, first and second portions of blood can be collected from a plurality of blood donors, and each second portion can be processed to produce a unit of platelet concentrate (PC). A plurality of units of PC can subsequently be pooled.

A variety of pooling arrangements are suitable for carrying out the invention, and the invention is not to be limited thereby. Figure 3 illustrates an embodiment of a biological fluid pooling system that can be used in accordance with the invention. The illustrated system 100 includes a plurality of containers 18a, each suitable for holding a biological fluid such as PC, in fluid communication with a pooling assembly 141. In the illustrated embodiment, the pooling assembly 141 includes a network or plurality of conduits 140 that converge into a single conduit 60 at outlet or junction 50. The outlet or junction 50 of the pooling assembly 141 is in fluid communication with a receiving or transfer container 118a. In the illustrated embodiment, fluid communication with the receiving container 118a is provided by a conduit 60. Interposed in the conduit 60 between the outlet or junction 50 and the container 118a may be at least one device or assembly. For example, as shown in the illustrated embodiment, the pooling system 100 may include a gas inlet 80, a drip chamber 81, a filter assembly 26 such as a leukocyte depletion assembly, and a gas outlet 82. One example of a suitable system including a pooling assembly is disclosed in U.S. Patent No. 5,364,526.

Figure 4B illustrates another embodiment of a biological fluid pooling system

that can be used in accordance with the invention. The illustrated system includes a plurality of containers 12, each suitable for holding a biological fluid such as buffy coat, in fluid communication via conduits 160 with a first receiving container 118a for pooled buffy coat. The illustrated system in Figure 4B also includes a filter assembly 26, such as a leukocyte depletion assembly, interposed between the first receiving container 118a and a second receiving container 118a. Typically, the system also includes an additional container 20, e.g., for holding an additive or wash solution.

Figure 4A illustrates an embodiment of a biological fluid processing system 10 than can be used to produce the individual units of buffy coat that can be pooled using the system illustrated in Figure 4B. The system illustrated in Figure 4A includes a phlebotomy device 1, and a plurality of containers, e.g., containers 12, 18b, and 20 in fluid communication via a plurality of conduits 160, as described with respect to Figure 1. For example, containers 12 in Figure 4B may be comprised of individual units of buffy coat from container 12 in Figure 4A.

The containers 12, 18a, 18b, 20, and 118a may be constructed of any material and shape compatible with a biological fluid. A wide variety of these containers are already known in the art. For example, blood collection and satellite bags are typically made from plasticized PVC, e.g., PVC plasticized with dioctylphthalate (DOP), diethylhexylphthalate (DEHP) (e.g., di-(2-ethylhexyl) phthalate)), or trioctyltrimellitate (TOTM) (e.g., tri (2-ethylhexyl) trimellitate). Illustrative containers include, but are not limited to, those produced in accordance with UK Patent Application GB 2,301,822A, and U.S. Patent Nos. 4,280,497 and 4,670,013.

In an embodiment, at least one of the containers is made from PVC plasticized with tri 2-ethylhexyl trimellitate (TOTM). Illustratively, the container can be plasticized with at least about 30 weight percent TOTM. Typically, containers plasticized with TOTM also include, for example, at least one epoxidized vegetable oil, a metal soap, and/or mineral oil. Containers can be plasticized with a blend of plasticizers, e.g., TOTM and dioctylphthalate (DOP). The containers can provide for killing and/or preventing the reproduction of undesirable material such as microorganisms and/or viruses.

In some embodiments, e.g., involving the storage of a platelet-containing biological fluid such as platelet concentrate, such bags can provide a bacteriostatic or

bacteriocidal effect. The bags can provide the effect on bacteria that, for example, were present in the blood donor's blood stream before donation, and/or bacteria that contaminated the fluid during collection or storage.

One example of a suitable container is a CLX® bag, available from Medsep Corporation (Covina, CA).

In some embodiments, at least one of the containers is compatible with a biological fluid additive and/or preservative solution. Alternatively, or additionally, at least one of the containers is compatible with, for example, an antibacterial and/or antiviral agent. The agent(s) can be utilized to kill and/or prevent the reproduction of undesirable material such as microorganisms and/or viruses present with platelets, plasma, and/or red blood cells. Suitable agents are known in the art. Exemplary agents include, but are not limited to, quinolones and their derivatives, e.g., a quinolone carboxylic derivative such as ciprofloxacin.

The conduits 30-37, 140, 60, and 160 used in the instant invention may be constructed of any material compatible with biological fluid. Preferably, they may be composed of a flexible material, such as plasticized PVC, e.g., as described above with respect to the blood collection and satellite bags.

The flow control device 40 illustrated in the Figures comprises a clamp, seal, valve, transfer leg closure, or the like. Systems typically include a plurality of flow control devices, and they can be located within or on the conduits and/or the containers.

The filter assembly 26 illustrated in Figures 1, 3, and 4 comprises a housing including an inlet and an outlet, and defining a flow path between the inlet and the outlet, with at least one porous medium interposed between the inlet and the outlet. In a more preferred embodiment, the filter assembly 26 comprises a leukocyte depletion device, and the porous medium comprises a leukocyte depletion medium. The filter assembly 26 can be suitable for leukocyte depleting an individual unit of biological fluid (Figure 1), a plurality of units of biological fluid (Figure 3), or pooled units of biological fluid (Figure 4B).

In some embodiments, e.g., involving the leukocyte depletion of platelet-rich-plasma (PRP), pooled or non-pooled buffy coat, or transition zone material, the leukocyte depletion device comprises a combined leukocyte depletion medium/red cell barrier medium.

Systems according to some embodiments of the invention include a plurality of filter assemblies 26, e.g., to filter different components of the biological fluid. For example, in a variation of the embodiment illustrated in Figure 1, a second filter assembly 26 can be interposed between containers 12 and 20. For example, a second filter assembly 26 can be used to deplete leukocytes from a red blood cell containing biological fluid such as packed red blood cells (PRC).

Exemplary filter assemblies, particularly exemplary leukocyte depletion devices and media include but are not limited to those disclosed in U.S. Patent Nos. 5,152,905, 4,925,572, 4,880,548, 5,399,268, 5,217,627, and 5,100,564, as well as International Publication No. WO 93/04763.

Systems according to the invention can be open, or, more preferably, closed. As used herein, the term "closed" refers to a system that allows the collection, processing, filtration, storage, and preservation of donor blood or blood components without the need to enter the system (and risk contamination of the system). A closed system can be as originally made, or result from the connection of system components using what are known as "sterile docking" devices. Illustrative sterile docking devices are disclosed in U.S. Patent No. 4,507,119.

In some embodiments of the invention, the system can include additional elements or components, such as one or more additional containers, a drip chamber, at least one venting device, e.g., at least one gas inlet, at least one gas outlet, and/or at least one gas collection and displacement loop.

Illustratively, a gas inlet can be disposed upstream of a filter assembly such as a leukocyte depletion device, and/or a gas outlet can be disposed downstream of the filter assembly. For example, a gas inlet and/or a gas outlet may be used to maximize the recovery of biological fluid in receiving or transfer container 118a. Using the illustrative system illustrated in Figure 3 for reference, the gas inlet 80 and the gas outlet 82 may be, respectively, upstream and downstream of the filter assembly 26.

In accordance with the embodiment exemplified in Figure 3, gas inlet 80 is disposed downstream of the outlet 50 of the pooling assembly 141, and upstream of a drip chamber 81, which is upstream of the filter assembly 26. Gas outlet 82 is disposed downstream, interposed between the filter assembly 26 and the receiving or transfer container 118a. Alternatively, a gas inlet and/or a gas outlet may be positioned in a drip

chamber, a conduit, or the receiving and/or source containers.

The gas inlet and gas outlet each comprise at least one porous element designed to allow gas to pass therethrough. The gas inlet and gas outlet should be chosen so that the sterility of the system is not compromised. A variety of materials may be used, provided the requisite properties of the porous element are achieved. These properties include the necessary strength to handle the differential pressures encountered in use and the ability to provide the desired filtration capability while providing the desired permeability without the application of excessive pressure. In a closed system, the porous elements of the gas inlet and the gas outlet should also preferably have a pore rating of about 0.2 micrometer or less to preclude bacteria entering the system.

Preferably, the gas inlet and gas outlet include at least one liquophobic porous element. Because the liquophobic porous element is not wettable, or poorly wettable, by the biological fluid being processed in the system, gas in the system that contacts the liquophobic element will pass through it, while the biological fluid will not. The gas outlet may include at least one liquophilic porous element, that allows gas to pass through. In an embodiment, the gas outlet includes both a liquophobic membrane and a liquophilic membrane, and gas will pass through both membranes until the liquophilic membrane is wetted by the biological fluid. Additionally, the gas inlet and/or the gas outlet may be included in a housing, which may include a cap or closure.

Exemplary venting devices, including gas inlets, gas outlets, and/or gas collection and displacement loops, and processes for using them, are as disclosed in, for example, International Publication Nos. WO 91/17809 and WO 92/07656, and U. S. Patent Nos. 5,126,054, 5,364,526, and 5,472,621.

The processing of biological fluid in the context of the present invention may take place at any suitable time, which may be soon after donation. For example, when the biological fluid is donated blood, it is typically processed as soon as practicable in order to maximize the number of components derived and to maximize blood component viability and physiological activity. Early processing may more effectively reduce or eliminate contaminating factors, including, but not limited to, leukocytes and microaggregates. In accordance with the subject invention, the biological fluid may be processed within about 24 hours of collection from the donor. The subject invention may also include processing biological fluid in accordance with United States practice,

wherein the processing of whole blood is generally within 8 hours of collection from the donor.

In accordance with the invention, leukocyte depletion can be carried out on any blood component at any point in the processing protocol. For example, leukocyte depletion can be carried out while separating platelet-rich-plasma (PRP) from red blood cells, while pooling platelet concentrate (PC), while filtering pooled or non-pooled buffy coat, or while administering PC.

An exemplary embodiment of a method according to the invention can be described with reference to Figures 1, 2, and 4, which illustrate a phlebotomy device 1 having first and second phlebotomy needles 2 and 3, wherein the device 1 is in fluid communication with multiple containers, e.g., a multiple blood bag system including a blood collection bag 12, and one or more satellite bags, e.g., 20, 18a, and 18b. Typically, blood collection bag 12 contains an anticoagulant, and satellite bag 20 contains an additive such as a red cell storage solution.

Flow control devices 40 such as clamps (associated with conduits 31 and 32) are initially closed, and needles 2 and 3 are initially capped (caps not shown).

A blood donor's arm is prepared for venipuncture in the usual manner, and needle 2 is uncapped and inserted into the donor's vein. Needle 3 is uncapped and inserted into a blood sampling device such as a vacutainer, the clamp 40 associated with conduit 31 is opened, and a first portion of biological fluid is passed into the vacutainer. In some embodiments, the needle 2 cuts a disc of skin from the donor, and the skin plug can pass into the vacutainer.

One or more blood sampling devices can be sequentially filled as desired. After the last sampling device is filled, clamp 40 associated with conduit 31 is closed, and the needle 3 is removed from the vacutainer. Clamp 40 associated with conduit 32 is opened, and a second portion of biological fluid is passed into collection container 12. This portion of biological fluid is less likely to present a significant risk of bacterial contamination during storage. After a suitable volume of biological fluid is collected in the container, flow control device 40 is closed, and needle 2 is removed from the donor. In some embodiments, after needle 2 and/or 3 is uncapped, or removed from the donor, the needle is placed in a device such as a phlebotomist protector to minimize the risk of accidental needle puncture.

Typically, the unit of biological fluid (i.e., the second portion of biological fluid) in the container 12 is processed to separate one or more blood components.

For example, the unit of biological fluid can be processed to form concentrated red blood cells and platelet-rich-plasma (PRP), and the PRP is processed to produce platelet concentrate (PC) and plasma. Alternatively, the unit of biological fluid can be processed to form concentrated red blood cells, buffy coat, and platelet-poor-plasma (PPP), and the buffy coat is subsequently processed to produce PC.

Illustratively, the container 12 can be centrifuged to form a sediment layer including red blood cells, and a supernatant layer including platelets suspended in plasma such as platelet-rich-plasma (PRP). In one embodiment, using the exemplary system illustrated in Figure 1 for reference, the PRP is passed through a filter assembly 26 comprising a leukocyte depletion device, and leukocyte-depleted PRP is collected in satellite container 18a. In some embodiments, the leukocyte depletion device comprises a leukocyte depletion/red cell barrier medium, and the leukocyte-depleted PRP collected in satellite container 18a is substantially free of red blood cells.

Typically, satellite containers 18a and 18b are subsequently separated from the rest of the system while maintaining a closed system. The leukocyte-depleted PRP can be further processed to form PC and plasma in containers 18a and 18b as is known in the art. If desired, the separated red blood cells, PC, and/or plasma can be stored until needed. In an embodiment, the method includes storing the separated components while killing and/or preventing the reproduction of bacteria and/or viruses that may be present in the fluid. For example, one or more containers, e.g., 12, 20, 18a and/or 18b may provide a bacteriostatic and/or a bacteriocidal effect. Alternatively, or additionally, at least one antibacterial agent and/or antiviral agent can be added to the containers(s), before or after the components are passed into the bag. If desired, the added agent(s) can be removed from the blood components before transfusing the components.

The separated components, e.g., stored or non-stored PC and/or plasma, can be pooled before further use.

In another illustrative embodiment, the PRP is not filtered. Rather, the PRP is processed to further separate the blood components, and the separated components can be filtered, e.g., as illustrated in Figures 2 and 3.

For example, the non-filtered PRP can be processed to produce PC and plasma

using the embodiments of the system shown in Figure 2. Subsequently, the PC can be filtered, e.g., while pooling multiple units of PC (as shown in Figure 3), or while administering individual units of PC to a patient (not shown). Of course, the PC (individual units or pooled units) can be stored until needed as described above. For example, the method can include storing the separated components while killing and/or preventing the reproduction of bacteria and/or viruses that may be present in the fluid.

In accordance with another embodiment of the invention, using the exemplary system illustrated in Figure 4A for reference, the second portion of biological fluid can be collected in the container 12 using phlebotomy device 1 as described above. Container 12 can be centrifuged to form a sediment layer including red blood cells, an intermediate layer including the majority of the platelets (the buffy coat), and a supernatant layer including most of the plasma such as platelet-poor-plasma (PPP).

The layers are separated (e.g., leaving buffy coat in container 12), and the buffy coat is processed to produce a platelet-containing blood product such as PC. If desired, multiple units of buffy coat can be pooled before producing the platelet-containing blood product. Illustratively, units of buffy coat can be pooled using the system illustrated in Figure 4B, or using a system similar to that illustrated in Figure 3, with or without a filter assembly 26 interposed between the pooling assembly 141 and the receiving container 118a. As noted above, these systems can be open or closed.

With respect to the embodiment illustrated in Figure 4B, individual containers 12 having buffy coat therein can be placed in fluid communication with each other, preferably by sterile docking the upstream conduit 160 from one container to the downstream conduit 160 from another container. Typically, the processing system also includes at least one receiving or transfer container 118a suitable for holding the pooled units of buffy coat, and a container 20 suitable for holding an additive solution such as a wash solution to wash buffy coat from one or more containers 12 (and conduits therebetween) into the first receiving or transfer container 118a. The buffy coats can be pooled as is known in the art.

The pooled buffy coat in container 118a can be centrifuged to form a sediment layer including red blood cells and a supernatant layer including platelets suspended in plasma. The supernatant layer can be filtered through a filter assembly 26 such as a leukocyte filter device, which can include a combined leukocyte depletion medium/red

cell barrier medium.

One embodiment of method using the system illustrated in Figure 3 includes introducing air or gas into source containers 18a prior to passing the biological fluid from the containers and through the pooling assembly. For example, air or gas may be introduced into the containers 18a through the gas inlet assembly 80 or the gas outlet assembly 82, preferably by using a syringe (not shown). The introduced air or gas is preferably ambient air or a sterile gas.

Introducing gas into the source containers 18a (Figure 3) may be accomplished by opening a flow path from the gas inlet 80 or the gas outlet 82 to the appropriate container 18a, while closing the flow path to the receiving or transfer container 118a. For example, the clamps on the conduits leading to the receiving or transfer container 118a and all but one container 18a may be closed, so that when gas is introduced into the system, gas in the conduit will enter the open container. In one embodiment, the process includes introducing gas sequentially into the containers 18a. The flow path to each source container may be closed after gas has been introduced into that container.

The flow path from the gas inlet 80 or the gas outlet 82 is then closed. The flow path to the first container 18a is then opened, and as the biological fluid passes from the first container 18a, and flows through the pooling assembly 141 toward receiving or transfer container 118a, it displaces the gas that was ahead of the column of flowing biological fluid. If desired, this gas can be exhausted or removed from the system. The gas may be vented, e.g., through an open gas outlet 82. Once the gas has been vented, the gas outlet may be inactivated, e.g., to prevent gas from entering the system. The gas outlet may include both a liquophobic element and a liquophilic element, which inactivates the outlet automatically, upon wetting the liquophilic element with the biological fluid.

Once the gas ahead of the biological fluid column has been exhausted and the flow of biological fluid has stopped, clamps 40 adjacent to the other containers 18a are opened, preferably, sequentially, so that biological fluid from the other containers 18a may pass through the pooling assembly 141 (Figure 3) toward the receiving or transfer container 118a. The clamp 40 adjacent to the receiving or transfer container 118a is opened so that the biological fluid can flow into the container 118a. Preferably, the clamp 40 adjacent to the receiving or transfer container 118a is opened before the clamps

adjacent to the other source containers are opened.

Initiating the flow of biological fluid from the other source containers also displaces gas ahead of the other units of biological fluid. Preferably, this gas may be collected in drip chamber 81 interposed between the outlet or junction 50 and the receiving or transfer container 118a. Passing the biological fluid through a drip chamber 81 may include collecting gas and/or controlling the rate of flow of the biological fluid. The drip chamber 81 is typically inverted until the biological fluid fills the drip chamber, at which point the drip chamber is returned to its normal orientation.

In accordance with an embodiment of the invention as illustrated in Figure 3, the biological fluid may also be passed through a filter assembly 26 such as a leukocyte depletion device interposed between the outlet or junction 50 of the pooling assembly 141 and the receiving or transfer container 118a. Preferably, the filter assembly 26 is located between the gas inlet 80 and the gas outlet 82.

As the biological fluid passes through the drip chamber 81 and the optional filter assembly 26, the gas ahead of the biological fluid may be passed through the gas outlet 82 as described previously. Pooled biological fluid is then recovered in the receiving or transfer container 118a and, in accordance with the invention, the introduction of air or gas into the receiving container can be minimized, so the biological fluid is recovered without collecting excess air.

In order to maximize recovery of biological fluid, gas may be introduced behind the biological fluid retained in the system. Using the illustrative system illustrated in Figure 3 for reference, the gas that was initially introduced into the containers 18a through either the gas inlet 80 or the gas outlet 82 will follow the biological fluid as it flows through the conduits. This increases the recovery of the biological fluid, since the gas following the biological fluid "chases" the fluid from the conduits. Furthermore, after the biological fluid has passed through the pooling assembly into the receiving or transfer container 118a and the containers 18a have collapsed, gas may be introduced behind the retained biological fluid by opening gas inlet 80. Additional biological fluid may then be recovered in the receiving or transfer container 118a.

Once recovery of biological fluid has been completed, receiving or transfer container 118a may be sealed and separated from the system, without the introduction of air into the container. Preferably, receiving or transfer container 118a is heat sealed,

although other methods of sealing are also suitable.

Further embodiments are encompassed by the present invention. For example, biological fluid can be collected without separating a first portion, and the biological fluid can be filtered and placed in a container that has a bacteriocidal or bacteriostatic effect on bacteria that may be present in the biological fluid. For example, a unit of whole blood can be collected, and centrifuged to form packed red blood cells and platelet-rich-plasma (PRP). The PRP can be passed through a leukocyte depletion device and the leukocyte depleted PRP, or platelet concentrate (PC) derived therefrom, can be placed in a container comprising polyvinyl chloride (PVC) plasticized with tri (2-ethylhexyl) trimellitate (TOTM).

All of the references cited herein, including publications, patents, and patent applications, are hereby incorporated in their entireties by reference.

While the invention has been described in some detail by way of illustration and example, it should be understood that the invention is susceptible to various modifications and alternative forms, and is not restricted to the specific embodiments set forth. It should be understood that these specific embodiments are not intended to limit the invention but, on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

We claim:

1. A method for processing biological fluid comprising:

obtaining a first portion of biological fluid;

obtaining a second portion of biological fluid; and

passing at least one component of the second portion of biological fluid through a leukocyte depletion medium.

2. The method of claim 1, including centrifuging the second portion of biological fluid to form a sediment layer and a supernatant layer, and passing the supernatant layer through the leukocyte depletion medium.

3. The method of claim 1, further comprising collecting at least one component of the second portion of biological fluid in a container downstream of the leukocyte depletion medium, said container being plasticized with tri (2-ethylhexyl) trimellitate.

4. The method of any one of claims 1-3, wherein the biological fluid comprises platelet-containing fluid.

5. The method of claim 1, wherein obtaining the first portion of biological fluid includes passing the first portion into a blood sampling device.

6. The method of claim 1, wherein obtaining the first portion of biological fluid includes obtaining a skin plug-containing biological fluid from a biological fluid donor; and wherein obtaining the second portion of biological fluid includes obtaining a skin plug-free biological fluid from the biological fluid donor.

7. A method for processing biological fluid comprising:

A. obtaining a first portion of biological fluid from a first source of biological fluid;

obtaining a second portion of biological fluid from the first source of biological fluid;

B. obtaining a first portion of biological fluid from a second source of

biological fluid;

obtaining a second portion of biological fluid from the second source of biological fluid; and

C. combining at least one component of the second portion of biological fluid from the first source of biological fluid with at least one component of the second portion of biological fluid from the second source of biological fluid to produce pooled biological fluid.

8. The method of claim 7, wherein obtaining the second portion of biological fluid from the first source of biological fluid includes passing the second portion into a first flexible container, and obtaining the second portion of biological fluid from the second source of biological fluid includes passing the second portion into a second flexible container;

the method further comprising centrifuging the second portions of biological fluid in the first and second flexible containers to form a sediment layer and a supernatant layer in each of the first and second flexible containers.

9. The method of claim 8 further comprising passing the supernatant layer from the first flexible container through a first leukocyte depletion device to provide a first leukocyte-depleted supernatant layer, and passing the supernatant layer from the second flexible container through a second leukocyte depletion device to provide a second leukocyte-depleted supernatant layer.

10. The method of claim 9, further comprising processing the first leukocyte-depleted supernatant layer to provide a first unit of platelet concentrate, and processing the second leukocyte-depleted supernatant layer to provide a second unit of platelet concentrate.

11. The method of claim 7, further comprising passing the pooled biological fluid through a leukocyte depletion device.

12. The method of any one of claims 1-11, carried out in a closed system.

13. The method of any one of claims 1-6, further comprising storing at least one component of the second portion for about two days or more.

14. The method of any one of claims 7-11, further comprising storing the pooled biological fluid for about two days or more.

15. The method of any one of claims 1-6, wherein obtaining a first portion of biological fluid comprises collecting a essentially anticoagulant-free biological fluid from a donor, and wherein obtaining a second portion of biological fluid comprises combining the second portion with an anticoagulant.

16. A method for processing biological fluid comprising:

obtaining a unit of biological fluid;

centrifuging the biological fluid to form a sediment layer including red blood cells and a supernatant layer including platelets;

passing the supernatant layer through a leukocyte depletion device to provide leukocyte-depleted supernatant layer;

centrifuging the leukocyte-depleted supernatant layer to provide concentrated platelets and platelet-poor-plasma;

separating the platelet-poor-plasma from the concentrated platelets; and

storing the concentrated platelets in a container for at least about 24 hours, the container comprising a plasticized bag, the bag manufactured from a film including tri (2-ethylhexyl) trimellitate as a plasticizer.

17. A system for processing biological fluid comprising:

a phlebotomy device including at least two needles, wherein at least one needle is suitable for penetrating the skin of a biological fluid donor;

a leukocyte depletion device in fluid communication with the phlebotomy device.

18. The system of claim 17 further comprising at least a first blood bag and a second blood bag, wherein the leukocyte depletion device is interposed between the first blood

bag and the second blood bag.

19. The system of claim 17 or 18 wherein the second blood bag comprises a plasticized bag, the bag manufactured from a film including tri (2-ethylhexyl) trimellitate as a plasticizer.

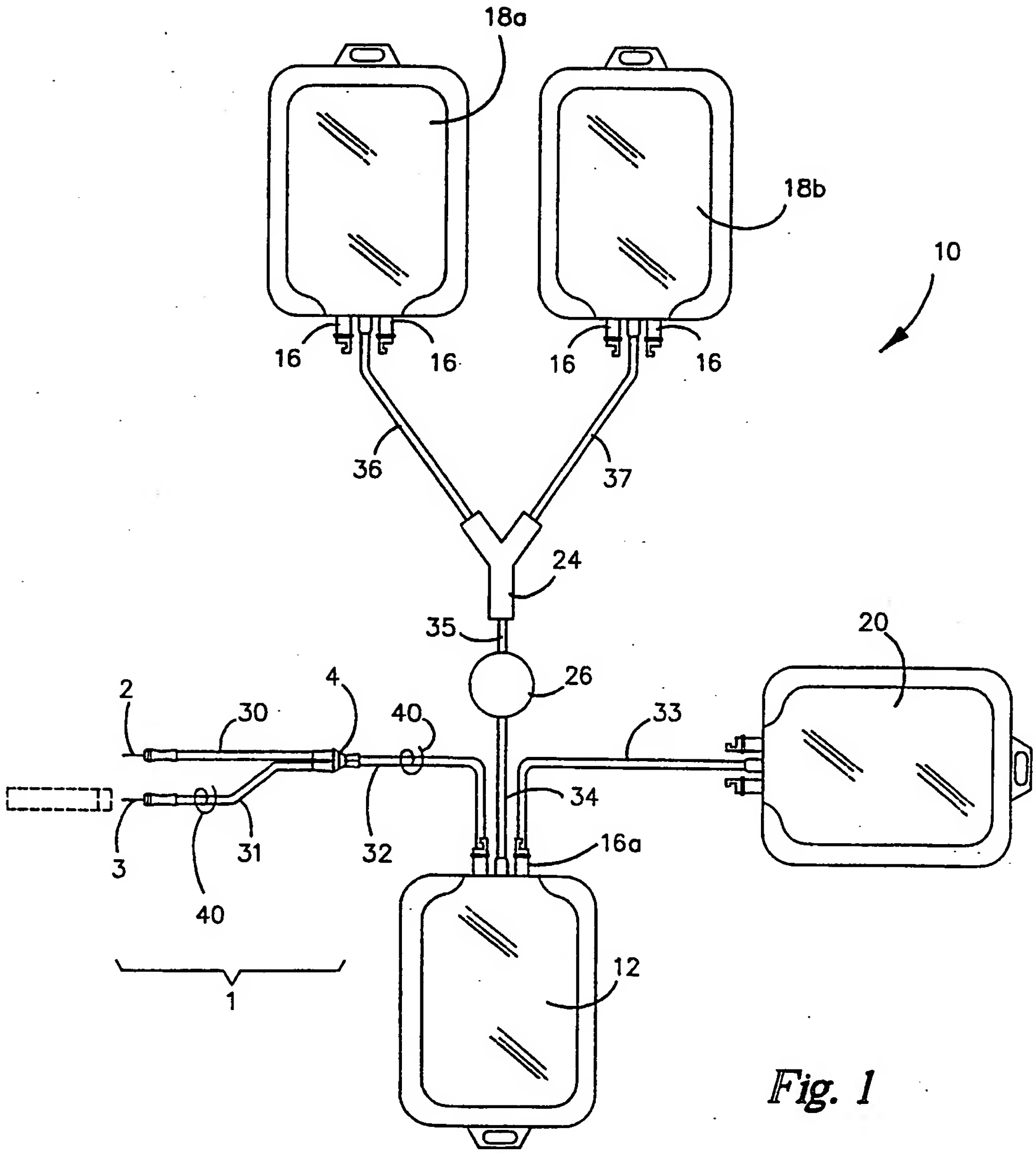
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Fig. 1

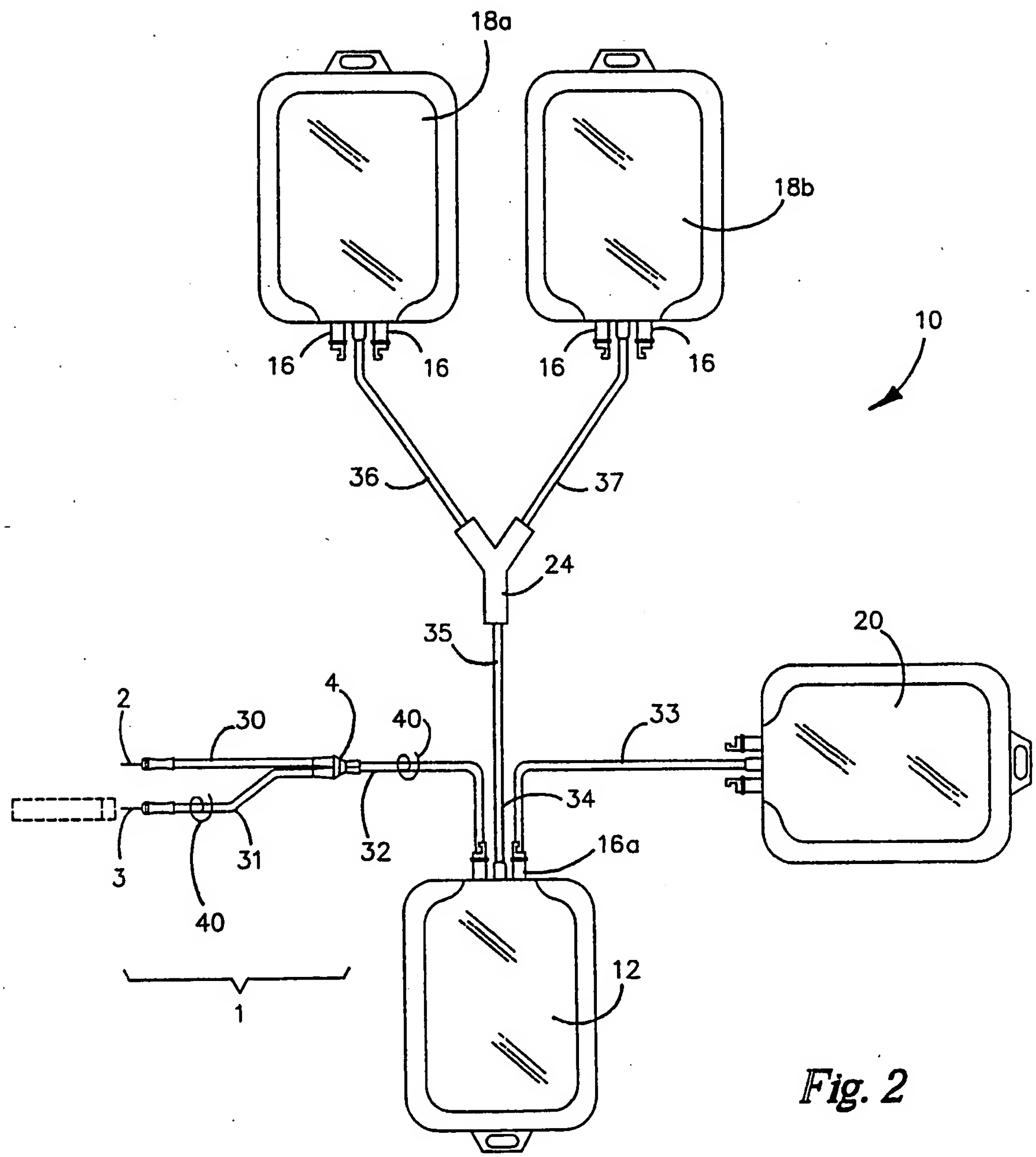
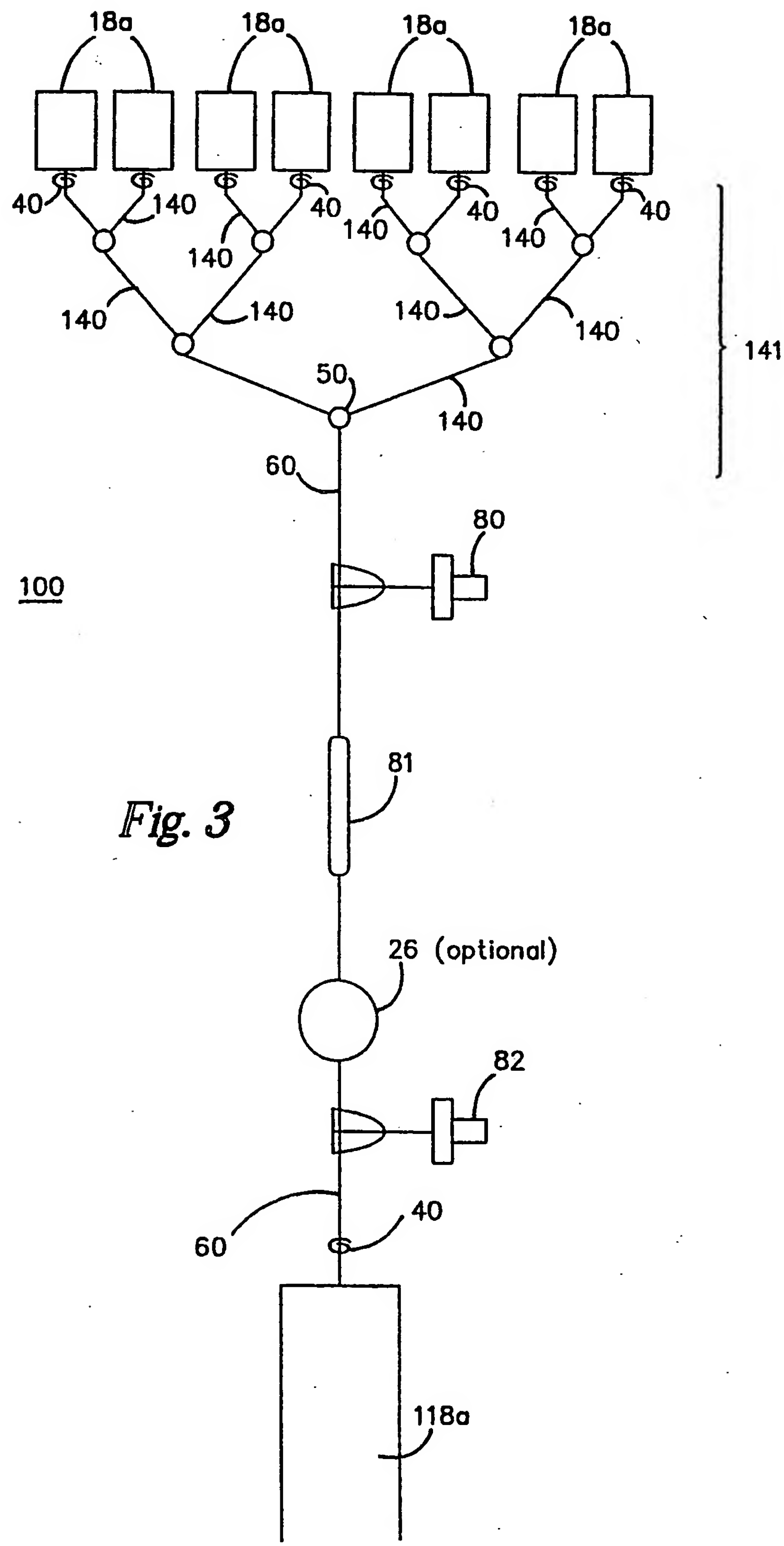



Fig. 2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26 (optional)
82
60
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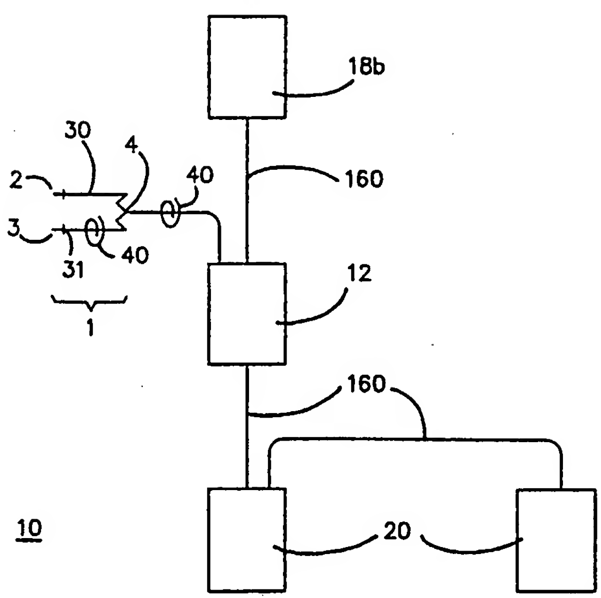
118a

Fig. 3

*Fig. 4*



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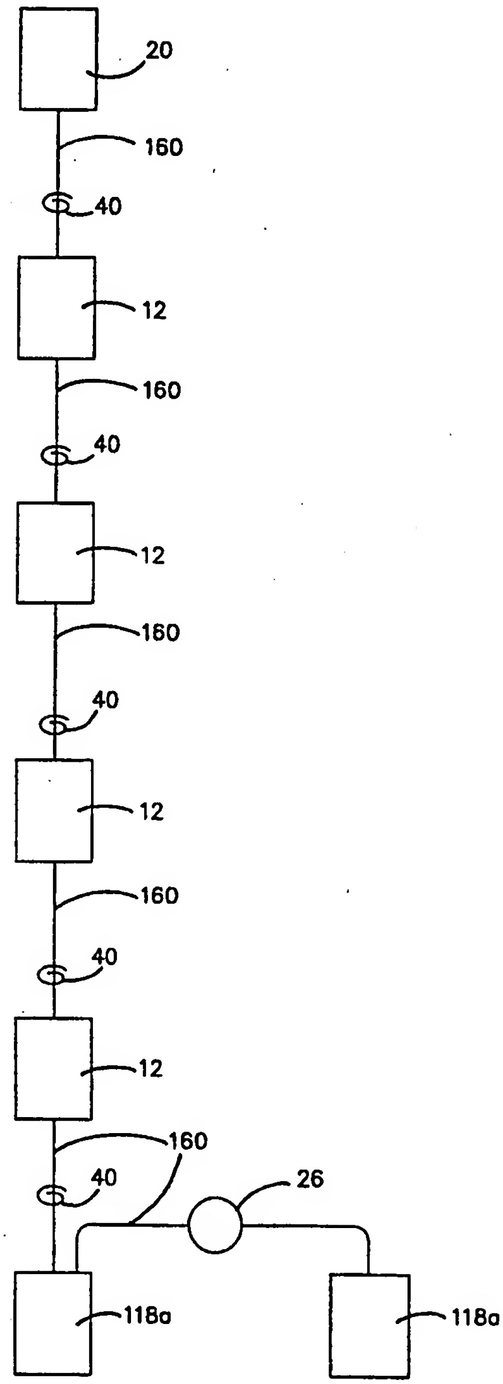

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## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23558

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :B01D 21/26, 36/00; A61M 1/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/206, 257.1, 258, 435, 767, 782, 787, 789, 805, 806; 604/ 4, 5, 6, 30, 406, 408, 410

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | US 5,364,526 A (MATKOVICH ET AL) 15 November 1994 (15.11.94), see entire document | 1<br>---<br>4, 17-19 |
| X<br>--<br>Y | US 4,985,153 A (KURODA ET AL) 15 January 1991 (15.11.91), see entire document. | 1-2<br>---<br>3-4, 17-19 |
| X<br>--<br>Y | US 5,472,621 A (MATKOVICH ET AL) 05 December 1995 (05.12.95), see entire document. | 7, 11<br>---<br>8-10, 12 |
| X<br>--<br>Y | US 5,100,564 A (PALL ET AL) 31 March 1992 (31.03.92), see entire document. | 1<br>---<br>3-4, 7-16 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 MARCH 1998

Date of mailing of the international search report
20 APR 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23558

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | US 5,549,834 A (BROWN) 27 August 1996 (27.08.96), see entire document. | 1<br>----------------<br>2-4, 8-19 |

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

210/206, 257.1, 258, 435, 767, 782, 787, 789, 805, 806; 604/ 4, 5, 6, 30, 406, 408, 410